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Published
With international search report.

(54) Title: A VACCINE AGAINST CHOLESTEROL TO PREVENT HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS

(57) Abstract

The present invention relates to immunoreactive compositions and methods for immunizing humans or animals against cholesterol and more particularly to the use of these compositions in methods for reducing the serum cholesterol levels of the immunized individuals and to retard or reduce the severity of atherosclerosis or atherosclerosis plaques caused by ingestion of dietary cholesterol.

+ See back of page

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

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A Vaccine Against Cholesterol to Prevent Hypercholesterolemia And Atherosclerosis

I. GOVERNMENT INTEREST

The invention described herein may be manufactured, licensed and used by or for governmental purposes without the payment of any royalties to us thereon.

II. RELATED APPLICATION(S)

This application is a continuation-in-part of U.S. Patent Application Serial No. 07/444,214 filed December 1, 1989, which in turn is a continuation-in-part of U.S. Patent Application Serial Number 06/875,048 filed June 2, 1988. Additionally, the application is a continuation-in-part of U.S. Patent Application Serial No. 07/601,090 filed October 22, 1990, which in turn is a continuation-in-part of U.S. Patent Application Serial No. 07/202,599 filed June 2, 1988 now U.S. Patent No. 4,885,256 issued December 5, 1989.

III. FIELD OF THE INVENTION

This invention relates to immunoreactive compositions for immunizing or hyperimmunizing humans against cholesterol and more particularly to the use of these compositions in methods for reducing the serum cholesterol levels of the immunized individuals and to retard or reduce the severity of atherosclerosis or atherosclerosis plaques caused by ingestion of dietary cholesterol or by other factors.

IV. BACKGROUND OF THE INVENTION

It is widely believed that high levels of serum cholesterol are a significant causative effect in the pathogenesis of atherosclerosis and associated diseases such as atherosclerotic coronary heart disease, atherosclerotic cerebral vascular disease, renal disease, etc. It is also believed that lowering of blood cholesterol levels is associated with amelioration of atherosclerotic vascular diseases (Goodman, D.S. et al., Report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Arch. Intern. Med. 148:36-69, 1988; Kromhout, D. et al., Serum cholesterol and 25-year incidence of and mortality from myocardial infraction and cancer. The Zutphen Study. Arch. Intern. Med. 148:1051-1055, 1988). In 1984, a National Institutes of Health consensus development conference panel recommended a framework of detection and treatment of hypercholesterolemia. Based on this the National Cholesterol Education Program has made the well-known recommendation to adults: "Know your cholesterol number" (Luepker, R.V. et al., Recommendations regarding public screening for measuring blood cholesterol. Summary of a National Heart, Lung, and Blood Institute Workshop, October 1988. Arch. Intern. Med. 149:2650-2654, 1989).

The major methods recommended for achieving reduced serum cholesterol levels are through reduction of dietary intake of cholesterol and other fats, and treatment of hypercholesterolemic individuals with drugs designed to lower blood cholesterol. The blood cholesterol levels are particularly associated with homeostatic mechanisms related to levels of plasma lipoproteins that serve as carriers of cholesterol. The dangerous lipoproteins, from the standpoint of atherosclerotic risk are the low density lipoproteins (LDL), and the levels of LDL are regulated by the functional activities of LDL receptors on the surfaces of cells, particularly in the liver (Brown, M.S. and Goldstein, J.L. A receptor-mediated pathway for cholesterol homeostasis. Science 232:34-47, 1986). Many of the strategies for using drugs to reduce blood cholesterol involve interference with the processing of cholesterol derived from LDL (Brown and Goldstein, 1986).

The extent that cholesterol can be reduced by diet is limited by numerous factors, and the reduction of cholesterol by drugs could be associated with side effects of the drugs as well as cost. In any case, a variety of additional variables can influence cholesterol levels, such as genetic background, stress effects, and age. Additional methods for reduction of cholesterol might be expected to have beneficial health effects, particularly in individuals who might receive such treatment before significant progression of atherosclerotic disease has occurred.

The present invention describes the use of a vaccine formulation that would be used to immunize humans against cholesterol and thereby lower the concentration of serum cholesterol, either by itself or in combination with other methods commonly used to lower cholesterol. A variety of immunization procedures might be used to induce antibodies to cholesterol, and the presence of antibodies to cholesterol would be detected either by a solid-phase immunoassay using crystalline cholesterol or a cholesterol conjugate or by a complement-dependent assay such as complement-dependent immune damage to liposomes containing cholesterol as taught by Swartz et al. [Antibodies to cholesterol. Proc. Nat. Acad. Sci. 85:1902-1906, 1988] and Alving et al. [U.S. Patent No. 4,885,256 issued 5 December 1989].

To our knowledge, humans have never been actively immunized against cholesterol and the safety of doing this, as well as the potential consequences relating to serum cholesterol levels or progression of atherosclerosis due to intake of dietary lipids, has not been established. It has been demonstrated that human sera usually do contain varying quantities, depending on the individual serum, of "naturally-occurring" antibodies to cholesterol [Alving et al., Naturally occurring autoantibodies to cholesterol in humans. Biochem. Soc. Trans. 17:637-639 (1989)]. However, there has not been any correlation of such naturally-occurring antibodies with serum cholesterol levels or with atherosclerosis.

The possibility has been discussed that naturally-occurring antibodies to cholesterol might be a normal part of the aging process and might contribute to (rather than ameliorate) atherosclerosis (Alving, C.R.

Antibodies to liposomes, phospholipids, and cholesterol: Implications for autoimmunity, atherosclerosis, and aging. In: Horizons in Membrane Biotechnology, Nicolau, C. and Chapman, D., editors, Wiley-Liss, pp. 41-41, 1990). The possible dangers of injecting liposomes containing cholesterol into animals containing antibodies to cholesterol have been illustrated by anaphylactoid effects observed by Wassef et al. [Anaphylactoid reactions mediated by autoantibodies to cholesterol in miniature pigs. J. Immunol. 143:2990-2995 (1989)]. Therefore it is not obvious that this invention could have practical use in humans. Nonetheless, the potential feasibility of this invention as a possible safe and effective vaccine against cholesterol has been demonstrated by experiments in humans in which repeated injections of a candidate liposomal anti-malarial vaccine that contained cholesterol did result in the production of antibodies to cholesterol. This is clearly indicated in a U.S. Patent Application Serial No. 07/601,090 entitled: "Encapsulated High-Concentration Lipid A Compositions as Immunogenic Agents To Produce Human Antibodies To Prevent Or Treat Gram-negative Bacterial Infections" by Alving and Swartz filed on 22 October 1990. In that disclosure, the example shown in Figure 9 therein clearly demonstrates that antibodies to cholesterol can be safely induced in certain individuals. The present invention utilizes an antigen that produces higher and more consistent antibodies than in the previous anti-malarial disclosure, and produce such antibodies for the purpose of preventing diet-induced serum cholesterol elevations and atherosclerosis.

Although we have not found in the prior art any teaching relating to immunization of humans with cholesterol, in the literature there has been one attempt described to try to ameliorate hypercholesterolemia and atherosclerosis in rabbits by immunological means. Bailey et al. [Immunization with a synthetic cholesterol-ester antigen and induced atherosclerosis in rabbits. Nature 201:407-408 (1964)] immunized rabbits with an antigen consisting of cholesterol conjugated to bovine serum albumin. Bailey et al. stated that the "mean antibody titre measured by an interfacial precipitation technique was 1:7000", but there was no attempt to produce or to measure antibodies that had specificity against cholesterol. The assay antigen consisted of the original conjugate, not cholesterol either

alone or as part of another conjugate. At no place did Bailey et al. teach that they had induced antibodies to cholesterol, and they did not teach that antibodies to cholesterol could have been produced or that such antibodies might have played a role in the lowering of serum cholesterol levels or amelioration of atherosclerosis.

Bailey et al. did observe a reduced hypercholesterolemia and less aortic plaque formation in the immunized animals that were fed a cholesterol-rich diet. However, in the absence of further information the antibody titer could have been entirely directed against the bovine serum albumin component and the cholesterol-albumin conjugate might simply have lowered cholesterol through nonspecific mechanisms, such as by nonspecific adsorption or serum cholesterol by the albumin. This latter explanation could be supported by the fact that albumin has a considerable degree of hydrophobicity and can be used as a reagent to promote solubility of cholesterol in an aqueous medium such as serum. The disclosure by Bailey et al. is too insufficient to draw any immunological conclusion regarding the role, if any, that antibodies to cholesterol may have played in the experimental results. It is probably because of this that Bailey et al. did not teach any such role.

V. SUMMARY OF THE INVENTION

This invention consists of a vaccine which is effective in immunizing humans against cholesterol. The purpose of this would be to reduce the serum cholesterol levels of the immunized individuals and to retard or reduce the severity of atherosclerosis or atherosclerosis plaques caused by ingestion of dietary cholesterol or by other factors. The vaccine would consist of a formulation containing cholesterol or cholesterol and phosphatidyl choline; or cholesterol and dimyristoyl phosphatidyl choline together with a suitable delivery vehicle and may also contain a suitable adjuvant. The relative molar ratio between the cholesterol and phosphatidyl choline or dimyristoyl phosphatidyl choline is within the range of 1:1 to 1:2.5. An example of a suitable formulation would be liposomes containing phosphatidylcholine, cholesterol, and lipid A in molar ratios of liposomes containing phosphatidylcholine, cholesterol, and lipid A in molar ratios of

2/5/0.02 (where the molarity of lipid A is based on the molarity of phosphate in native lipid A). This ratio is not necessarily critical, because other ratios might be successful in accomplishing the same result. Delivery vehicles other than liposomes would also be suitable, including microcapsules, microspheres, lipospheres, polymers, and slow release devices could serve instead of liposomes. An experiment in rabbits has demonstrated that the stipulated vaccine does ameliorate diet-induced elevations of serum cholesterol.

VI. BRIEF DESCRIPTION OF THE DRAWING

A more complete appreciation of the invention and many attendant advantages thereof will be readily obtained by reference to the following DETAILED DESCRIPTION OF THE INVENTION when considered in conjunction with the accompanying drawing, wherein:

Figure 1 shows IgG responses 2 weeks after initial immunization in the 6 human volunteers that constituted Group IV in the experimental immunization cholesterol from the above patent application. The individuals were immunized with 43% cholesterol liposomes as taught in the present disclosure and in the previous patent application. Each of the components was individually tested by ELISA for the appearance of IgG antibodies against the purified individual component. In the case of lipid A, the individuals were injected with liposomes containing monophosphoryl lipid A. The data are shown with preimmunization values, if any, subtracted from the postimmunization values. Each serum was diluted 1/100 for ELISA analysis. Three of the six immunized individuals developed significantly increased levels of antibodies to cholesterol. Figure 1 corresponds to Figure 9 from the U.S. Patent Application Serial No. 07/601,090, entitled: "Encapsulated High-Concentration Lipid A Compositions as Immunogenic Agents To Produce Human Antibodies To Prevent Or Treat Gram-negative Bacterial Infections" by Alving and Swartz filed on 22 October 1990.

VII. DETAILED DESCRIPTION OF THE INVENTION AND EXAMPLES

The working example set forth below illustrate, without any implied limitation a vaccine useful for the immunization of humans against cholesterol. This vaccine is useful for immunizing or hyperimmunizing a human against cholesterol, which vaccine comprises as an active ingredient A. a delivery vehicle and B. either, (i) cholesterol; or (ii) cholesterol and an adjuvant; or (iii) cholesterol, phosphatidyl choline on an adjuvant; or (iv) cholesterol, dimyristoyl phosphatidyl choline and an adjuvant; or (v) cholesterol and phosphatidyl choline; or (vi) cholesterol and dimyristoyl phosphatidyl choline.

EXAMPLE

An experiment is currently underway to determine the possible feasibility of ameliorating diet-induced hypercholesterolemia and atherosclerosis in rabbits. Groups of rabbits are being immunized while other groups are not being immunized against cholesterol; at least one group of immunized and one group of nonimmunized rabbits will be fed a diet rich in cholesterol. It is our prediction that the immunization process will ameliorate the hypercholesterolemia and atherosclerosis that is expected to be produced by the cholesterol-rich diet and that this will reduce to practice the invention that is herein disclosed. The experimental results from the rabbit experiment described below provides substantive evidence in support of our prediction by demonstrating that the 1% cholesterol diet causes a dramatically increased serum cholesterol level within 1 week (6 weeks after immunization in those rabbits that were immunized), and the cholesterol continues to rise over the second week (7 weeks after initial immunization was started in the immunized animals). However, the increased level of diet-induced cholesterol was the 30% less elevated in the animals (Group II) that were immunized against cholesterol (see the Table herein).

Experimental Diets

At week 6, the experimental diets were initiated. The diets consisted either of ordinary rabbit chow or a 1% cholesterol diet (obtained from Bioserve). Four groups and two subgroups of animals were employed: Group I, 4 rabbits, not immunized, fed normal diet; Group IIa, 4 rabbits, immunized intramuscularly, fed 1% cholesterol diet; Group IIb, 2 rabbits, immunized intravenously, fed 1% cholesterol diet; Group III, 4 rabbits, not immunized, fed normal diet; Group IVa, 4 rabbits, immunized intramuscularly, fed normal diet; Group IVb, 2 rabbits, immunized intravenously, fed normal diet.

In addition to the above teaching with rabbits, it is now evident that even liposomes containing 43% cholesterol (using liposomes as taught above and in the prior art described above) also can induce antibodies to cholesterol in a limited number of individual humans.

It appears that the 71% cholesterol liposomes will be superior to the 43% cholesterol liposomes as the basis of a vaccine to induce antibodies to cholesterol. This conclusion is drawn from the fact that only a small number of the individual humans immunized with liposomes containing 43% cholesterol developed antibodies to cholesterol [see Fig. 1, which is derived from the previous U.S. Patent Application Serial No. 07/601,090, entitled: "Encapsulated High-Concentration Lipid A Compositions as Immunogenic Agents To Produce Human Antibodies To Prevent Or Treat Gram-negative Bacterial Infections" by Alving and Swartz filed on 22 October 1990, the latter of which is a continuation-in-part of U.S. Patent Application Serial No. 07/202,509 filed June 2, 1988 (A Vaccine For Induction of Immunity to Malaria)]. The contrast, approximately 70% of spleen cells from mice immunized with 71% cholesterol were producing antibodies to cholesterol [Swartz et al., Antibodies to cholesterol. Proc. Nat. Acad. Sci. 85:1902-1906, 1988] and Alving et al., [U.S. Patent No. 4,885,256 issued 5 December 1989].

Based on the prior art it is evident that cholesterol is highly immunogenic and the immunogenicity is enhanced both by adjuvants (e.g.,

microliters of goat anti-mouse IgN (micro-chain) alkaline phosphatase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) at 1 microgram ml in PBS containing 10~ FBS was added to the wells and incubated 1 hour at room temperature. Plates were again washed three times for 5 minutes each PBS. Fifty microliters of the substrate, p-nitrophenyl phosphate at 2 mg/ml in diethanolamine buffer (Kirkegaard and Perry Laboratories) was added to the well and incubated 30 minutes at room temperature. Plates were scanned for optical activity at 405 nm using a Titertek Multiscan (Flow Laboratories). Values reported were adjusted by subtracting value in blank wells that lacked both antigen and monoclonal antibody.

Immunization Protocol

Four groups of rabbits were either immunized with liposomes containing 71 mol% chol, or were not immunized. Immunization was performed either intramuscularly or intravenously every two weeks for 6 weeks. The immunization procedure routinely induced antibodies to cholesterol in rabbits, as determined by ELISA or by complement-induced immune damage to high-cholesterol liposomes as taught by Swartz et al., Antibodies to cholesterol. Proc. Nat. Acad. Sci. 85:1902-1906, 1988, and Alving et al., U.S. Patent No. 4,885,256 issued 5 December 1989.

The immunization of human subjects with 43% cholesterol liposomes was conducted as part of the testing of the efficacy of a vaccine for induction of antibodies to malaria antigen and antibodies to lipid A, as taught by the previous disclosure entitled: "Encapsulated High-Concentration Lipid A Compositions as Immunogenic Agents To Produce Human Antibodies To Prevent Or Treat Gram-negative Bacterial Infections" by Alving and Swartz that is currently being prepared as a U.S. patent application, the latter of which is a continuation-in-part of U.S. Patent Application Serial Number 07/202,509 filed June 2, 1988 (A Vaccine For Induction of Immunity to Malaria. Anti-cholesterol antibodies induced were detected in Group IV and are illustrated in the accompanying Figure.

METHODS

Liposomes

Liposomes are being manufactured by standard methods in which liposomes loaded with cholesterol (containing 71% cholesterol) and also containing lipid A as an adjuvant are prepared for injection as taught by Swartz et al. [Antibodies to cholesterol. Proc. Nat. Acad. Sci. 85:1902-1906, 1988] and Alving et al. [U.S. Patent No. 4,885,256 issued 5 December 1989].

The liposomes used for immunization contained dimyristoyl phosphatidylcholine (DMPC)/cholesterol (chol)/dimyristoyl phosphatidyl glycerol (DMPG)/lipid A (molar ratio 0.9/2.5/0.1/0.02 for rabbits, or 0.9/0.75/0.1/0.02 for humans, where the molarity of lipid A refers to lipid A phosphate). The total dose of lipid A injected as part of the 71% cholesterol liposomes was 50 ug lipid A. The liposomal cholesterol concentration is described as a percentage, and this is calculated as mol % with reference to (DMPC + DMPG); e.g., a cholesterol/(DMPC + DMPG) ratio of 0.75/1 is 43 mol%, and 2.5/1 is 71 mol%.

Enzyme-linked Immunosorbent Assay (ELISA).

ELISAs were performed by using crystalline cholesterol as an antigen on the bottoms of the wells of microtiter plates. Chrystalline cholesterol was coated onto the surface of wells in polystyrene plates (Immunlon 96, "U" bottom, Dynatech Laboratories, Alexandria, VA) by addition of an ethanolic solution and evaporation of the solvent by air under a fume hood. Places were further dried under high vacuum and stored at -30°C when not used the same day. Plates were blocked by addition of phosphate-buffered saline (PBS: 137 mM NaCl/2.7mM KCl/9.6mM phosphate, pH7.2) containing 10% heat-inactivated fetal bovine serum (FBS) (M.A. Bioproducts, Walkersville, MD). This was accomplished by washing the wells three times for 10 min each. Fifty microliters of ascites fluid containing monoclonal antibodies, diluted in PBS containing 10% FBS, was added to the wells and incubated 1 hr at room temperature. Plates were then washed three times for 5 minutes each with PBS. Fifty

lipid A or other adjuvants) and by the epitope density of cholesterol used for immunization. It should be possible to achieve the combination of high epitope densities of cholesterol together with adjuvants by a variety of carrier mechanisms, including microcapsules, microspheres, lipospheres, high density conjugation or association of cholesterol with proteins or other macromolecules, natural sources of high cholesterol (such as organisms such as mycoplasma that have the capacity to accumulate cholesterol). It is presumed that any established method for inducing antibodies to particulate substances or macromolecules theoretically could be adapted to inducing antibodies to cholesterol.

Experimental Results

Table 1. Reduction of Diet-Induced Hypercholesterolemia in Rabbits Immunized Against Cholesterol.

Group *	High Cholesterol Diet**	Immunized ***	Bleeding Time (Weeks)	Serum Cholesterol (mg/dl)	Increase Compared to Week 5	Reduced Increase (%)
I	–	–	5	76		
II	–	+	5	62		
III	–	–	5	73		
IV	–	+	5	83		
I	+	–	6	775	699	
II	+	+	6	797	734	
III	–	–	6	64		
IV	–	+	6	68		
I	+	–	7	1205	1129	
II	+	+	7	952	790	30
III	–	–	7	74		
IV	–	+	7	62		

*Data shown are means of results (Group I, 4 rabbits; II, 6 rabbits; III, 4 rabbits; IV, 6 rabbits).

**The 1% cholesterol diet was initiated at the 5 week time point after starting the experiment.

***The immunization against cholesterol was initiated at 0 weeks.

The above results demonstrate that the high cholesterol diet invariably caused elevated serum cholesterol values. However, two weeks after initiating the diet (week 7) the elevation of cholesterol in the immunized group (Group II) was 30% less than the elevation of cholesterol in the nonimmunized group (Group I).

We Claim:

1. A vaccine for immunizing or hyperimmunizing a human against cholesterol, which vaccine comprises as an active ingredient

A. a delivery vehicle and

B. either;

(i) cholesterol; or

(ii) cholesterol and an adjuvant; or

(iii) cholesterol, phosphatidyl choline and an adjuvant; or

(iv) cholesterol, dimyristoyl phosphatidyl choline and adjuvant; or

(v) cholesterol and phosphatidyl choline; or

(vi) cholesterol and dimyristoyl phosphatidyl choline.

2. A vaccine according to Claim 1 wherein the adjuvant is lipid A.

3. A vaccine according to Claim 1 wherein the delivery vehicle is selected from the group consisting of biocompatible-biodegradable, or biocompatible-nonbiodegradable liposomes, or polymers; slow release devices; and combinations thereof.

4. A vaccine according to Claim 3 wherein the delivery vehicle is a liposome or polymer.

5. A vaccine according to Claim 4 wherein the delivery vehicle is a polymer.

6. A vaccine according to Claim 4 wherein the delivery vehicle is a liposome.

7. A vaccine according to Claim 4 wherein the delivery material is in the form of microcapsules.

8. A vaccine according to Claim 4 wherein the delivery material is in the form of microspheres.

9. A vaccine according to Claim 2 wherein the delivery material is a slow-release device.

10. A vaccine according to Claim 1 consisting essentially of phosphatidyl choline, cholesterol and a delivery vehicle.

11. A vaccine according to Claim 1 consisting essentially of phosphatidyl choline, cholesterol, an adjuvant and delivery vehicle.

12. A vaccine according to Claim 1 consisting essentially of phosphatidyl choline, cholesterol and a delivery vehicle which contains lipid A.

13. A vaccine according to Claim 1 consisting of dimyristoyl phosphatidylcholine, cholesterol and a delivery vehicle.

14. A vaccine according to Claim 1 consisting essentially of dimyristoyl phosphatidyl choline, cholesterol, an adjuvant and a delivery vehicle.

15. A vaccine according to Claim 1 consisting essentially of cholesterol and a delivery vehicle.

16. A vaccine according to Claim 1 consisting essentially of cholesterol, an adjuvant and a delivery vehicle.

17. A vaccine according to Claim 3 consisting essentially of dimyristoyl phosphatidylcholine, cholesterol and a delivery vehicle which contains lipid A.

18. A vaccine according to Claim 1 wherein the relative molar ratio between the cholesterol and phosphatidyl choline or dimyristoyl phosphatidyl choline is within the range of 1:1 to 1:2.5.

19. A vaccine according to Claim 18 wherein the relative molar ratio between the phosphatidyl choline and cholesterol is 1:2.5.

20. A vaccine according to Claim 18 wherein the relative molar ratio between the phosphatidylcholine, cholesterol and lipid A is 1:2.5:0.02.

21. A vaccine according to Claim 18 wherein the relative molar ratio between the dimyristoyl phosphatidylcholine and cholesterol is 1:2.5.

22. A vaccine according to Claim 18 wherein the relative molar ratio between the dimyristoyl phosphatidylcholine, cholesterol and lipid A is 1:2.5:0.02.

23. A therapeutic method for vaccinating a human against cholesterol to prevent hypercholesterolemia or atherosclerosis, said method comprising treating said human prior to the human having hypercholesterolemia or atherosclerosis caused by serum cholesterol, with an amount of the vaccine of Claim 1 to result in passive prophylaxis.

24. A therapeutic method for vaccinating a human having hypercholesterolemia or atherosclerosis caused by serum cholesterol, said method comprising treating said human with an amount of the vaccine of Claim 1 effective to result in the suppression of serum cholesterol or amelioration of atherosclerosis.

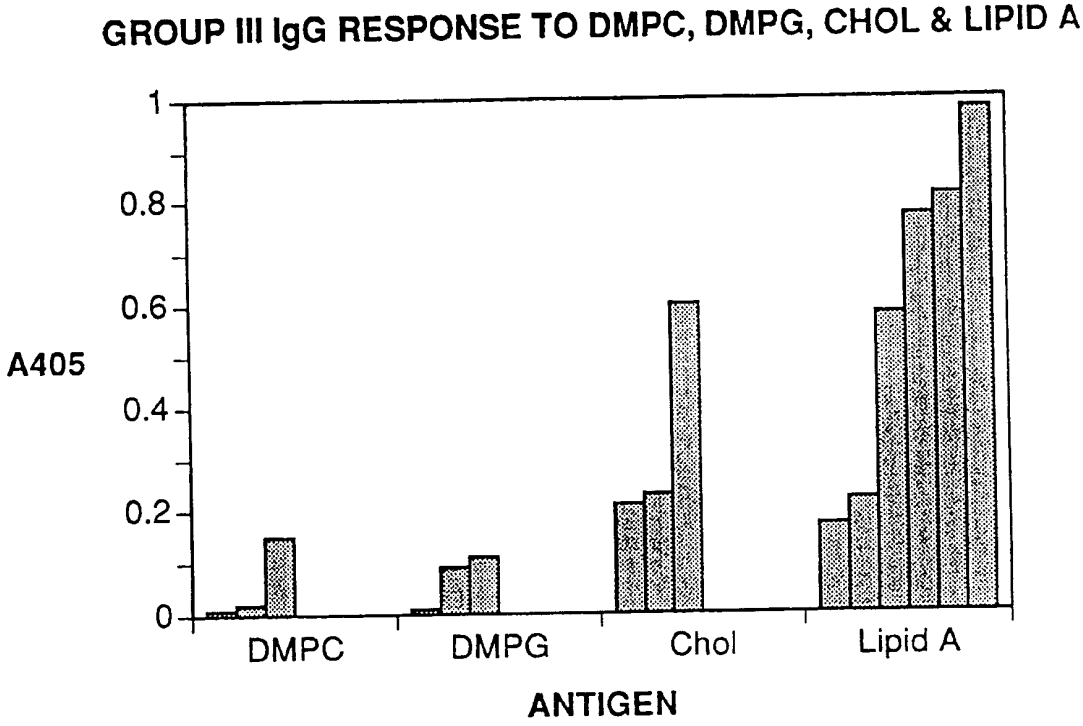
GROUP III IgG RESPONSE TO DMPC, DMPG, CHOL & LIPID A
A405
1
0.8
0.6
0.4
0.2
0
DMPC
DMPG
Chol
Lipid A
ANTIGEN

FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09268

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 37/22, 9/50, 31/59, 31/685
US CL : 424/88, 92, 450, 457, 468, 489; 514/964, 824, 724, 963

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	424/88, 92, 450, 457, 468, 489; 514/964, 824, 724, 963

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

APS File

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
X/Y	Proc. Natl. Acad. Sci., Volume 85, issued March 1988, Swartzeral, "Antibodies to Cholesterol", pages 1902-1906, see especially pages 1902 and 1903.	1-22/ 1-22
X/Y	Tom et al eds., "Liposomes and Immunobiology" published 1980 by Elsevier North Holland, Inc. (NY) see pages 67-78, especially page 72.	1-4,6-17/ 1-4,6-17
X/Y	Gregoriadis ed, "Liposome Technology", published 1984 by CRC Press (Boca Raton) see pages 157-175, especially page 171-172.	1-4,6-17/ 1-4,6-17
X/Y	Biochimica Biophysica Acta, Volume 689, issued 1982, Banerji et al., "Membrane Lipid composition Modulates the Binding Specifity of a monoclonal Antibody against Liposomes", pages 319-326, see especially page 320.	1-4,6-17/ 1-4,6-17
X/Y	Immunochemisty, Volume 9, issued 1972, Sato et al "Anti-Cholesterol Activity in antisera against human serum lipoproteins", pages 585-587, see especially pages 585-586.	1,3-5,7-11/ 15,16

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
24 FEBRUARY 1992	06 MAR 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	Kay K. Kim, Ph.D.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Nature, Volume 201, issued 25 January 1964, Bailey et al, "Immunization with a synthetic cholesterol-ester Antigen and induced Asherosclerosisin Rabbits", pages 407-408, see especially pages 407.	23,24
A	Science, Volume 237, issued 04 April 1986, Brown et al, "A receptor-Mediated Pathway for cholesterol Homeostasis", pages 34-47.	1-24

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.